PHENOLIC COMPOSITION OF THE PULP OF ARGAN (Argania spinosa L. Skeels)
FRUITS AND RELATION WITH THEIR MORPHOLOGICAL CHARACTERISTICS
[COMPOSITION PHÉNOLIQUE DE LA PULPE DES FRUITS D'ARGANIER (Argania spinosa L. Skeels) ET RELATION AVEC LEURS CHARACTERISTIQUES

MORPHOLOGIQUES]

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Skeels) ET RELATION AVEC LEURS CHARACTERISTIQUES MORPHOLOGIQUES

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SUMMARY. - Phenolic compounds of the Argan tree pulp were characterized. Their levels can be used to distinguish between four fruit forms: spherical, oval, oval-apiculated and spindle shaped fruits. The spindle shaped fruits are characterized by their higher phenolic content (7.2 mg/g fresh weight pulp (FW) in comparison with the spherical and oval forms containing 4.8 and 5.02 mg/g FW respectively. The oval-apiculated form seems to be midli rich in phenolic compounds (5.8 mg/g FW). The analysis of phenolic extracts by itPLC showed a quantitative difference of some phenolic compounds, particularly (-)-epicatechin, others unidentified flavons and quercetin derivatives. A positive correlation between total phenol content and phenotypic characters was established.

INTRODUCTION - The argan (Argania spinosa (L.) Skeels) is a member of the Sapotaceae endemic to southwestern Morocco. It currently covers an area of 820,000 hectares (AYYAD, 1989). forest species provides the users with significant revenue for its products (wood, forage, oil). It is a spiny tree, the appearance of which is reminiscent of the olive tree; its knotty trunk is often formed of several interlaced stems. Its leaves are small, lanceolate, persistent, leathery, and pale on the lower face. The argan tree flowers in May-June; the flowers are hermaphroditic, having a yellow-green color (MENSIER, 1957). The argan produces fruit from the age of 5 years (RAHMANI, 1979; CHARROUF, 1984). Its fruits are sessile berries consisting of a fleshy pericarp or pulp occupying 43% of the entire fruit and of a nut having a very hard shell (52.6%) which encloses 1 to 2 flattened kernels (4.4%). Its leaves and its pulp constitute a food very appreciated by animals. Its wood is also used as fuel. The kernels contain an oil called "Argan oil," used as such in human food. Its fatty acid composition

^{*} Numbers in margin indicate pagination of foreign text.

resembles that of olive oil with a little less oleic acid; however, the organoleptic properties are quite different (Huyghebaert and Hendrickx, 1974). Argan oil is not sold on the entire Moroccan market. It is produced by craftsmen and locally consumed. Its price is very high as compared with that of olive oil (Prendergast and Walker, 1992). Its richness in unsaturated fatty acids (more than 80%) gives it dietetic and nutritional qualities permitting its use in cosmetics and therapeutics (Olivier, 1987; Mattson, 1983). In addition to this economic contribution, the argan tree is one of the species best adapted to the severe conditions of arid and semi-arid regions and thus constitutes a potential means of combating desertification. Unfortunately, this forest species is subject to an intense shrinkage in area estimated at 600 hectares per year (Benzyane, 1985). This shrinkage in area which could entail its disappearance constitutes a menace both for the local population and for the environmental equilibrium. Therefore, the preservation of the argan tree has become a priority which demands initiating multidisciplinary research in order to increase our knowledge about this tree and to better evaluate its potentialities. The argan is a plant that has great genetic variability that deserves to be studied and characterized. It has phenotypic polymorphism at the level of the shape of its fruits (Metro, 1952; Ferradous et al., 1995). The variability also affects the biochemical characteristics, in particular the diversity of isoenzymatic systems, MDH, GOT, PH, and

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EST (Msanda et al., 1994) and the chemical composition of the oil (Maalah, 1992). Thus, different components can be used as markers in order to characterize the biodiversity, in particular the phenolic compounds. The latter have been used as taxonomic indicators in order to deal with the genetic variability of numerous plant species. is the case, for example, of the date palm, where numerous cultivars have been characterized by their pool of glycosylated flavonoids (Ouafi, et al., 1988). The same is true of the apple (Ilzarabe et al., 1991) and the olive (Vlahov, 1992). These compounds can also be evaluated on the phytochemical level. Up to now, the investigation of the phenolic content of the pulp has not particularly been concerned with the phenotypic variability of argan fruits. Our work was carried out for the purpose of studying the phenotypic variability of argan fruits having different morphological natures (spherical, oval, oval-apiculated, and fusiform) originating from a population from the region of Essaouira by: analysis of the biometric parameters of the fruits and seeds and by the investigation of the phenolic composition of the fruit pulp. The possible correlations between the biometric parameters of the fruits and their phenolic composition were investigated.

MATERIAL AND METHODS - 1) plant material and biometric parameters. The fruits were collected from a score of trees of an argan plantation in the Essaouira region. The sampled trees present a diversity of morphological characteristics. Four fruit shapes were

observed: spherical, oval, oval-apiculated, and fusiform. The fruits were collected in three physiological stages of development and maturation of the fruit:

Stage I: young fruits beginning to enlarge. Stage II: developed fruits beginning to mature. Stage III: completely ripened fruits.

30 fruits from each tree were characterized by their length (Lf), width (lf), weight (Pf), as well as the width/length ratio (l/L). The same measurements were made on the nuts (Ln, ln, and Pn). The weights of the kernels (Pa) were obtained after crushing the nuts.

2) Extraction and analysis of the phenolic compounds. The pulp of the fruits was ground and homogenized cold in a mortar in the presence of a methanol/water (80/20) hydroalcoholic mixture. The extraction and the purification of the phenolic compounds were performed according to the method described by Macheix et al. (1990) and El Hadrami, et al. (1997). The total polyphenol content was determined by the Folin Ciocalteux method (Macheix, 1974).

The compounds were separated by two-dimensional thin film chromatography (TFC) (butanol/acetic acid/water 4-1-2v/v, 2% acetic acid) on a cellulose plate with an aluminum support and without a fluorescence indicator. One-dimensional TFC (formic acid/toluene/acetone, 1/3/3) on a silicon plate was used for the separation of flavan polymers. These compounds have been made pink by vanillin - HCl (Sokar and Howarth, 1976). The phenolic compounds

have been characterized by their fluorescence under UV at 254 nm and 366 nm in the absence and presence of ammonia and by their reactions with chemical developers, in particular Benedickt's [sic] reagent (Reznik and Egger, 1961) and Neu's reagent (Andary, 1975). Flavonoid aglycones have been obtained by hydrolysis in an acid medium (HCl 2N) of the phenolic extract of the flavonoids isolated by TFC at 100°C for 1 hour (FRANCIS, 1982).

Analysis of the phenolic compounds by high performance liquid chromatography (HPLC) was performed on a C18 column of a Gilson (806 Manometric Model) type device, provided with a diode bar detector (Crystal 240) and coupled with a UV spectrophotometer which makes it possible to record the spectra of compounds eluted in the course of the analysis. The mobile phase consists of the doubly distilled acetonitril/water mixture acidified to pH 2.6. The HPLC chromatograms were obtained at three wave lengths 280 nm, 320 nm, and 350 nm, which correspond respectively to the absorption maxima of flavans, hydroxycinnamic derivatives, and flavonols.

The compounds were identified by their retention time and by their UV absorption spectrum in comparison with standards (Sigma Products). Three successive extractions were made on the same initial material, the variability connected with the analysis of the phenolic compounds did not exceed 6%. The results obtained were processed by variance analysis using the "Statitcf" software (Newman [sic. Translator's note: no close parenthesis] and Keuls Test with a

probability threshold (0.05%) and by principle component analysis (ACP) using the "Biomeco" program.

Table 1. Coefficients of correlation between the variable biometric characteristics and the principle components PC1, PC2, and PC3 for the four forms of argan fruits studied. The PC1, PC2, and PC3 axes represent respectively 69, 12.9, and 18% of the total variance.

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		 5 - 7 SPROPROSOCOCO 			
Variable	PCI	PC2	rc:		
L.f	0.83	0,02	0,00		
W	0,33	0.00	0,45		
Pf	0,28	0,39	0,12		
La	0.88	0,00	0,00		
In	0,28	0.08	0.28		
Pn .	0.61	0.00	0.01		
Pa	0.25	0.45	0.02		
M.	0,91	0,00	0,02		

RESULTS - 1) Principal component analysis (PCA). The first principle component (PC1) is strongly correlated with the length of the fruit (Lf) (0.83), the length of the nut (Ln) (0.88), to the ratio of the fruit (1/L) (0.91) and the weight of the nut Pn (0.61) (Table 1). This component explains 69% of the total variability. The second component (PC2) is correlated with the weight of the fruit (Pf) (0.39), and the weight of the kernel (Pa) (0.45). It explains 12.90% of the total variance. As for the third component (PC3), it is connected with the width of the fruit (1f) (0.45), and with that of the nut (ln) (0.28). It represents 18% of the total variance. The first and the third components combined present a percentage of variability of 87%. On the level defined by the two components, the four forms of fruits studied (see photograph) are principally

distinguished along the first axis (first principal component) and the third axis (third principal component) (Fig. 1). On the positive side of axis 1, the spherical shape is clearly distinguished from the other shapes. The oval shape is relatively nearer the spherical shape and intersects with the oval-apiculated shape. On the negative side, the fusiform shape overlaps with the oval-apiculated shape.

2) Analysis and characterization of phenolic compounds. The analyses of phenolic extracts of the fruits by TFC and by HPLC and their acid hydrolysis have made it possible to reveal three principal groups of phenolic compounds: flavan-3 ols, flavanols, and phenolic acids (Fig. 2).

The flavan-3 ols are represented by the (-)-epicatechin (peak 8), the (+)-catechin (peak 12), and other not yet identified oligomers corresponding to peaks 1, 2, 3, 5, 6, 7, 9, 10, 11, and 13 (Fig. 2).

Argan - Biometry

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EXTREME VALUES - 1.8739 6.8434 -0.9405 0.6799
REPRESENTATION OF 19 POINTS ON 1 PAGE (3) HORIZONTAL AXIS: 1 VERTICAL AXIS: 3

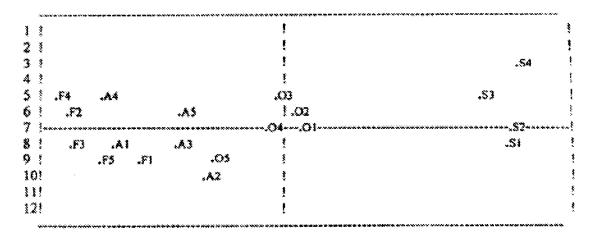


Fig. 1. Representation of different forms of argan fruits on the plane defined by the first axis (PC1) and the third axis (PC3).S: spherical shape; O: oval shape; A: oval-apiculated shape; F:

S: spherical shape; O: oval shape; A: oval-apiculated shape; F: fusiform shape.

These compounds are very abundant in the extracts of the argan pulp. Their proportion varies from 74.10% of the total polyphenols (for the oval-apiculated shape) to 80.60% (for the spherical shape).

The flavonols are represented by rutin (peak 14) and others derived from quercitin (peaks 15, 16, and 17). They are less dominant than the flavans. Their percentage with respect to the total polyphenols varies from 13.82% (for the spherical shape) to 16.50% (for the oval and oval-apiculated shapes).

The phenolic acids are represented by p-hydrobenzoic acid (peak 4) and the hydrocinnamic derivatives corresponding to peaks 18, 19, 20, 21, and 22. They are quantitatively less abundant than the other families. Their proportions with respect to the total phenols vary from 5.34% (for the spherical and oval fruits) to 9.4% (for the oval-apiculated fruits). The variance analysis indicates significant differences in total polyphenols between the three physiological stages of development and maturation of the fruit. The most important variations are recorded between stages I and II (Table 2). A decrease in the amounts of polyphenols passing from stage I (18.315 mg equivalent catechin/g of MF of the pulp in the case of the fusiform fruits) to stage III (7.281 mg equivalent catechin/g of MF

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of the pulp for these same fusiform fruits). The four shapes of the fruits studied also show significant quantitative differences in total polyphenols. Thus, the fusiform fruits have a higher polyphenol content (7.281 mg equivalent catechin/g of MF of the pulp in the case of ripe fruits) than the spherical and oval fruits (respectively 4.888 and 5.026 mg equivalent catechin/g of MF of the pulp). As for the oval-apiculated fruits, they are of average richness in polyphenols (5.893 mg equivalent catechin/g of MF of the pulp).

The reduction of the overall phenolic content in the course of the maturation process is particularly connected with the drop of the flavan content (compounds 6, 7, 9 and (-)-epicatechin) (Table 3). The most significant amounts of these compounds characterize the young fruits (respectively 2044, 1930, 1350, and 5480 µg/g of MF of the pulp in the case of the fusiform fruits), then decrease to reach relatively low levels in the ripe fruits (respectively 924, 816, 458, and 2,492 µg/g of MF of the pulp) (Table 3). The other principal phenolic compounds, p-hydroxybenzoic acid, rutin, compound 16 (flavanol) and compound 13 (flavan) follow a similar development but with a slight difference between the different stages of development and maturation of the fruit, in particular between stages II and III (Table 3).

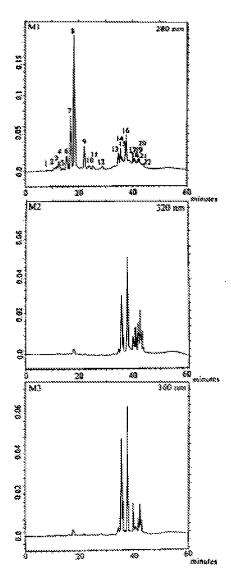


Fig. 2. — Chromatogrammes HPLC d'extrait phénolique de la puipe des fruits d'Arganier Flavas-3-ols: pies 1, 2, 3, 6, 7, 8 (-)-épicatéchine), 9, 10, 11, 12 ((+)-catéchine) et 13, Flavonols: pies 14 (Rudnel, 15 té et 17 Acides phénoliques: pies 1, 4 (acide p-kydroxybenzoique), 18, 19, 20, 21 et 22 (dérivés hydroxycinasmiques).

Fig. 2 - HPLC Chromatograms of the phenolic extract of the pulp of Argan fruits flavan-3-ols: peaks 1, 2, 3, 6, 7, 8 ((-)-epicatechin), 9, 10, 11, 12 ((+)-catechin) and 13, flavonols: peaks 14 (rutin), 15, 16 and 17 Phenolic acids: peaks 1, 4 (phydroxybenzoic acid), 18, 19, 20, 21 and 22 (hydroxycinnamic derivatives)

TABLE 2. Variation of the total polyphenol content (mg/g of MF of the pulp of different types of argan fruits as a function of the physiological stages of development and maturation of the fruit. The averages followed by the same letter are not significantly different at the probability threshold, a = 0.05%.

	Polyphenol c	ontent (mg/g	MF)
Fruit shape	Stage I	Stage II	Stage III
Spherical Oval Oval-apiculated Fusiform	10.110° ± 0.348 12.022° * 0.803 14.595° ± 0.583 18.315° ± 0.517	5,777 ± 0,339 6,627* ± 0,479 7,850c ± 0,308 8,564* ± 0,363	4,888° ± 0,291 5,026° * 0,139 5,893° ± 0,213 7,281° ± 0,143

The HPLC analysis of the phenolic extracts of the pulp of ripe fruits also showed a different quantitative distribution of certain phenolic compounds, in particular of the flavan-3-ols represented by (-)-epicatechin, compounds 6 and 9 (Table 4). In fact, these compounds are advantageously found in the fusiform shape (respectively 2492, 924, and 458 μ g/g of MF of the pulp) with respect to the oval-apiculated shape (respectively 1900, 388, and 337 μ g/g of MF of the pulp). However, the lowest amounts are found in the spherical (respectively 1547, 211, and 256 μ g/g of MF of the pulp) and oval (respectively 1740, 259, and 205 μ/q [sic] of MF of the pulp) shapes. The (+)-catechin does not show variation between the four shapes of fruits studied. This compound is present in low proportions in the phenolic extracts of the pulp of the argan fruits (0.5 to 0.9% of the total polyphenols). Likewise, compounds 7 (flavan) are found in different fruit shapes in relatively similar amounts (Table 4). As for flavans 10, 13, and 20 (hydrocinnamic

derivative), they are present in relatively large amounts in the fusiform fruits (respectively 125, 275, and 171 μ g/g of MF of the pulp).

TABLE 3. Variations of the principal phenolic components of the argan fruits during the different physiological stages of development and maturation of the fruit.

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Essence de traite		[] i dpiesoretine	[8]	Emperi 7	131	y an y y and a second	1331	Rine	Compense 18
	Stade	4,229	0,691	1,619	0,631	6,300	0.234	0,360	0,692
i Spherique	Stade	3,114	0.241	1,265	0,429	0,337	0,345	0,250	6,300
M 1 1	Stade I	(1 1,547	0,211	0,882	6,256	0,086	0,159	0,269	0,279
	Stade	3,293	1,100	1,557	0,676	0,510	0,305	1,0865	0,954
Ovsle	Stude	(8) 1,900	0,393	1,292	0,848	9,266	6,213	0.26%	0,368
_i	Stade I	B 1,740	0.239	0,982	0,208	9,337	6,138	0,273	0,372
	S480c	\$ 4,747	1,324	2,043	0.972	0.677	9.371	1,4854	1,166
m)vale apicalé	Stade	B 2,374	0,667	1,322	0,538	0,299	6,248	0,418	0,498
	Stage 1	9,900	0,388	0,941	6,337	9,210	0.143	0,359	0,413
	Stade	3,480	2,044	1,930	1,360	9,685	6,611	0,933	1,191
n Fusiforms	Static	8 3,160	0,891	1,533	9.578	9,280	6,337	9,590	0,814
	Stade I	8 2,492	8,974	0,816	0.438	9,264	0,275	8,423	0,480

Key: a) Phenolic compounds

- b) Fruit shapes
- c) (-)-epicatechin
- d) Compound 6
- e) Compound 7
- f) Compound 9
- g) p-hydroxy-benzoic acid
- h) Compound 13
- i) Rutin
- j) Compound 16
- k) Spherical
 - Stage I
 - Stage II
 - Stage III
- 1) Oval
 - Stage I
 - Stage II
 - Stage III
- m) Oval-apiculated
 - Stage I
 - Stage II
 - Stage III
- n) Fusiform
 - Stage I

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Stage II Stage III

TABLE 4. Amounts of phenolic compounds (μ g/g of MF of the pulp) of different shapes of ripe argan fruits (Stage III).

Shape of the fruits					
Phenolic compounds	Spherical	Oval	Oval-		Fusiform
(μg/g MF)			apicu	lated	
Compound 3	61° ± 15	85° ± 13	104° ± 14	138° ± 13	8
Compound 6	211 * 19	259° ± 23	388° ± 22	924° ± 3	1
Compound 7	882° ± 62	952° x 32	$940^{\circ} \pm 23$	815° ± Z	2
Compound 9	256° ± 18	205* ± 19	377° ± 20	458b ± 3	ì
P-hydroxybenzoic acid (+)-catechin (-)-epicatechin Rutin	86° ± 8 44° ± 13 1547° ± 144 200° ± 10	137° ± 14 36° ± 14 1746° ± 183 273° ± 28	210° ± 22 35° ± 19 1990° ± 60 359° ± 11	264° ± 3 59° ± 1° 2492° ± 2 422° ± 34	7 \$6
Compound 10 Compound 13 Compound 16 Compound 18 Compound 20	62° ± 16 15° ± 22 210° ± 26 60° 19 111° ± 25	30° ± 14 138° ± 26 273° ± 39 78° ± 14 121° ± 10	59° ± 13 144° ± 12 413° ± 13 256° ± 19 143° ± 22	125° ± 20 275° ± 26 480° ± 41 158° ± 17 171a ± 22	

With respect to rutin and compound 16 (flavonols), the presence of greater amounts is noted in fusiform (respectively 422 and 480 μ g/g of MF of the pulp) and oval-apiculated (respectively 359 and 413 μ g/g of the pulp) fruits as compared with the spherical (respectively 209 and 279 μ g/g of MF of the pulp) and oval (respectively 273 and 371 μ g/g of MF of the pulp) fruits, the amounts of which are relatively small.

It is also noted that p-hydroxybenzoic acid, compound 18 (hydrocinnamic derivative), and compound 3 (flavan) are found in a higher amount in the fusiform (respectively 264, 158, and 138 μ g/g of MF of the pulp) and oval-apiculated (respectively 210, 265, and 104 μ g/g of MF of the pulp) shapes as compared with the spherical

(respectively 86, 60, and 61 μ g/g of MF of the pulp) and oval (respectively 137, 78, and 85 μ g/g of MF of the pulp) shapes.

3) Relation between the biometric characteristics of the fruit and the total polyphenol content. CPA analysis of the biometric parameters and the amount of phenolic compounds made it possible to distinguish among the four types of fruits studied according to the first and the second principal component (PC1 and PC2) (Fig. 3).

Argan-Biometry and Phenolic Composition VALUES OF THE EXTREMES -1.8739 6.8349 -2.2119 1.9277 REPRESENTATION OF 19 POINTS ON 1 PAGE (8) HORIZONTAL AXIS: 1 VERTICAL AXIS: 2

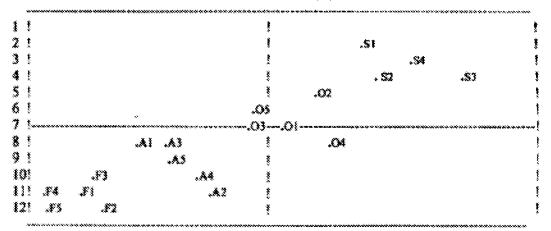


Fig. 3. Representation of different argan fruit shapes on the plane defined by the first axis (PC1) and the second axis (PC2) S: spherical shape; O: oval shape; A: oval-apiculated shape; F: fusiform shape

These two components represent 95.36% of the total variability. The first principal component (PC1) is strongly correlated with the total amount of polyphenols (0.87), the length of the fruit Lf (0.93), and the length of the nut Ln (0.93). Thus a good correlation between the phenolic compound content and the biometric parameters Lf (0.84) and Ln (0.83) is noted.

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polyphenols in the young argan fruits (stage I) has also been noted in numerous species of fruits, in particular in apples (MACHEIX, 1974) and olives (AMIOT, et al., 1986). It is evidence of an active phenolic synthesis during the first stages of the life of the fruit. The reduction of the phenolic content as a function of the maturity of argan fruits is explained by the physiological transformations during the maturation process. Concerning olives, Amiot et al., (1986) have reported that the reduction of the oleuropin and verbascoside contents is due to their reutilization by the cellular metabolism or their implication in the formation of anthocyans (compounds responsible for the maturation of the fruit).

The different shapes of the fruits studied displayed significant quantitative differences in phenolic compounds. Thus, the fusiform fruits are richer in total polyphenols and particularly in flavans ((-)-epicatechin) and other unidentified oligomeric shapes corresponding to peaks 6 and 9. These compounds make it possible to distinguish between three groups:

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Group A of fusiform fruits rich in flavans,

Group B of oval-apiculated fruits of average richness in flavans,

and Group C of oval and spherical fruits, relatively poor in flavans.

Quantitative differences in flavans have also been reported in

certain apricot varieties (Rich and Herrmann, 1988). The other phenolic compounds, in particularly the flavonols (rutin and compound 16) and the phenolic acids (p-hydroxybenzoic acid and compound 18) are found in higher and relatively close amounts in the fusiform and oval pointed fruits as compared with the spherical and oval fruits. These quantitative differences could be interesting for biochemical characterization of the phenotypic variability of argan fruits. In fact, these phenolic compounds have been used to characterize the varieties of certain plant species, in particular apricot (Rich and Herrmann, 1988; Garcia-Vigura, et al. 1994), apple (Ilzarbe et al, 1991), and olive (Vasquez-Roncer, et al., 1974; Vlahov, 1991).

Table 5 - Coefficients of correlation between the biometric characteristics (Lf, Ln and 1/L), content of polyphenols and the principal components PC1, PC2 and PC3. The axes PC1, PC2 and PC3 respectively represent 72.3, 23.06, and 4.67% of the total variance.

TABLEAU 5. – Coefficients de corrélation entre les caractères biométriques (Lf. Ln et UL), teneur en polyphénois et les composantes principales PC 1, PC2 et PC3 Les axes PC1, PC2 et PC3 représentent respectivement 72,3, 23,06 et 4,67% de la variance totale.

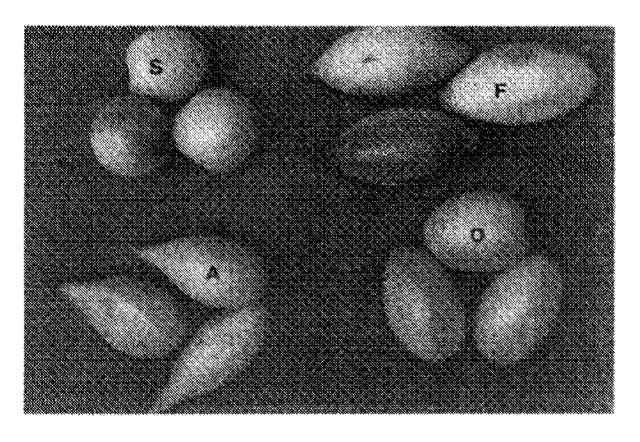
	Variables	PCI	PC2	PCI
	Lſ	0,93	0,01	0,05
	La	0,93	0,01	0,06
	1/L	0.02	0,98	0,00
a ke	ww en polypisénals	0,87	0,00	0,05

Key: a) Polyphenol content.

The phenolic compound content of argan pulp is positively correlated with the morphological characteristics of its fruits, in particular the length of the fruits and the nuts (Lf and Ln). A

similar result has been reported by Amiot, et al, (1986) in eight olive varieties. In fact, these authors have reported the presence of a correlation between the size of the fruit and the phenolic compound (oleuropein and verbascoside) content. The varieties that produce small olives are characterized by a high oleuropein and verbascoside content and the opposite is true of large-fruited varieties.

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PHOTOGRAPH. Illustration of different shapes of Argan fruits: S: spherical shape; O: oval shape; A- oval- apiculated shape; F: fusiform shape.

CONCLUSIONS. - This study has made it possible to characterize the principal phenolic compounds of argan fruits. It has also made it possible to reveal the presence of quantitative variations of these

compounds as a function of the stage of maturity and the shape of the fruit. The diversity of the morphological characteristics of argan fruits can be correlated with the variation of the phenolic composition of their pulp. These morphochemical criteria can be used in a complementary fashion in order to deal with the phenotypic or genetic diversity of argan fruits.

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